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A COMPARISON OF THE IMMUNIZING EFFECTS OF THE SUBCUTANEOUS AND INTRAPERITONEAL ADMINISTRATIONS OF TUMOR CELLS AGAINST THE GROWTH OF CARCINOMA IN MICE *

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In a previous communication[1] it was shown that the influence of a tumor on a second tumor, inoculated six days later, varied with the degree of so-called virulence of the tumor. The percentage of growing tumors was taken as the measure of virulence. The virulence was experimentally varied, in accordance with the method of Loeb, by exposing the tumor material in vitro to a temperature of 44 C. for varying periods of time. By this method, it was possible to use the same strain of tumor in all experiments and yet to have tumor material the virulence of which we could absolutely control. We found in these earlier experiments three types of growth after double inoculation: Simultaneous, or concomitant, growth, when both tumors grew in an approximately normal percentage of the animals (this was found when both tumors were virulent or both were of a medium degree of virulence); alternating (mutually exclusive) growth which occurred when two tumors of low virulence were inoculated, and in this case usually only one of the tumors grew, either the first or the second; inhibiting type noticed when a virulent, or medium virulent, tumor was inoculated first and a tumor of low virulence second, in which case the second tumor either did not grow or grew only in a relatively small percentage of the mice. The degree of the inhibition varied in direct proportion to the length of time the second tumor had been heated. There was also noted in these experiments a fourth type of growth, a stimulating type noticed when the growth of a tumor of medium or low virulence was possibly improved by the growth or inoculation of a tumor of medium virulence, and the beneficial effect appeared sometimes to affect the first,

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[1] Loeb: Centralbl. f. Bakteriol., 1912, 63, p. 450.

sometimes the second tumor. The existence of this type was at that time considered only probable as it had not yet been definitely proven.

This paper deals with the comparison of effects of subcutaneous and intraperitoneal tumor inoculations. In these experiments, the same adenocarcinoma of the mouse was used as in the former experiments[1] (Tumor 9 first transplanted by Loeb eight or nine years ago), when both the first and second tumors were inoculated subcutaneously.

THE GROWTH OF INTRAPERITONEAL TUMORS

The methods and technic used in these experiments were the same as used in the earlier experiments.[1] Before considering the results of our experiments on immunity it is necessary to compare the growth of a tumor inoculated intraperitoneally with that of one inoculated subcutaneously.

We find that unheated tumor material, or tumor material heated for either twenty-five or thirty minutes, grows in a smaller percentage of mice when inoculated intraperitoneally than when inoculated subcutaneously with material of similar virulence (Table 1). A heated or an unheated tumor, when inoculated intraperitoneally, grows in 60-70 percent of the animals in which similar material would grow if inoculated subcutaneously. When, however, five times the usual quantity of tumor material (375 mg.) is inoculated intraperitoneally, tumors develop in a slightly larger percentage of animals than after inoculation of the usual quantity. Intraperitoneal inoculation therefore is in our experiments less effective than subcutaneous inoculation. Therefore, our results regarding the percentage of growth of the intraperitoneal tumors differ from those of Krauss, Ranzi, and Ehrlich[2] and Paltauf.[3]

THE INFLUENCE OF A FIRST EITHER SUBCUTANEOUS OR INTRAPERITONEAL TUMOR ON A SECOND SUBCUTANEOUS TUMOR

When we compare the influence of a first, either intraperitoneal or subcutaneous, tumor on a second, subcutaneous tumor, inoculated six days later, we find that when an unheated tumor is inoculated first and either unheated tumor material or tumor material heated twenty-five minutes second, the immunizing effect of the less actively growing intraperitoneal (as compared to a subcutaneous) tumor is

[2] Ztschr. f. Immunitäts., 1910, 6, p. 665.
[3] Wien. klin. Wchnschr., 1909, 47, p. 1654.

greater than that of the first, subcutaneous tumor. The second, subcutaneous tumor grows in a smaller percentage of the animals when the first tumor is inoculated intraperitoneally than when inoculated subcutaneously. The effect of the first, intraperitoneal tumor on a second, unheated, subcutaneous tumor is the same as on a second, subcutaneous tumor heated either twenty-five or thirty minutes, but relatively the immunizing effect on the unheated tumor is greater.

TABLE 1

Percentage of Takes of the Various Tumors

Subcutaneous Tumor	Intraperitoneal Tumor	Both First (1) and Second (2) Tumors Inoculated Subcutaneously	First Tumor (1) Inoculated Subcutaneously, Second (2) Intraperitoneally	First Tumor (1) Inoculated Intraperitoneally, Second (2) Subcutaneously
75 mg. unheated 82 %	375 mg. unheated 62 % 78 mice	....	(1) 75 mg. unheated 51 % (2) 375 mg. unheated 27 % 47 mice	(1) 375 mg. unheated 55 % (2) 75 mg. unheated 25 % 57 mice
75 mg. unheated 82 %	75 mg. unheated 53 % 118 mice	(1) 75 mg. unheated 86 % (2) 75 mg. unheated 76 % 27 mice	(1) 75 mg. unheated 77 % (2) 75 mg. unheated 19 % 43 mice	(1) 75 mg. unheated 45 % (2) 75 mg. unheated 24 % 64 mice
75 mg. unheated 82 % 75 mg. heated 25′ 72 %	75 mg. unheated 53 % 75 mg. heated 25′ 43 % 37 mice	(1) 75 mg. unheated 67 % (2) 75 mg. heated 25 40 % 68 mice	(1) 75 mg. unheated 80 % (2) 75 mg heated 25′ 12 % 25 mice	(1) 75 mg. unheated 47 % (2) 75 mg. heated 25′ 22 % 45 mice
75 mg. unheated 82 % 75 mg. heated 30′ 55 %	75 mg. unheated 53 % 75 mg. heated 30′ 34 % 53 mice	(1) 75 mg. unheated 73 % (2) 75 mg. heated 30 21 % 37 mice	(1) 75 mg. unheated 84 % (2) 75 mg. heated 30′ 21 % 38 mice	(1) 75 mg. unheated 43 % (2) 75 mg. heated 30′ 20 % 41 mice
75 mg. heated 25′ 72 %	75 mg. heated 25′ 43 %	(1) 75 mg. heated 25′ 80 % (2) 75 mg. heated 25 60 % 28 mice	....	(1) 75 mg. heated 25′ 45 % (2) 75 mg. heated 25′ 30 % 54 mice
75 mg. heated 30′ 55 %	75 mg. heated 30′ 34 %	(1) 75 mg. heated 30 80 % (2) 75 mg. heated 30 26 % 21 mice	....	(1) 75 mg. heated 30′ 26 % (2) 75 mg. heated 30′ 61 % 74 mice

Thus, an unheated, subcutaneous tumor grows in 82 percent of the inoculated animals, but when it is inoculated six days after the inoculation of an intraperitoneal tumor, it grows in only 24 percent; a tumor heated twenty-five or thirty minutes grows subcutaneously in 72 percent or 55 percent of the animals, respectively, but when an intraperitoneal tumor has been inoculated first they grow in 22 percent and 20 percent of the animals, respectively. Thus, after the inoculation of an intraperitoneal tumor the heated, and unheated, subcutaneous tumors grow in nearly similar percentages, whereas normally the unheated tumor grows in a considerably larger number of inoculated animals than does the heated tumor. We find in these cases that the growth is concomitant in type; the second tumor grows only when the first tumor grows (Table 2).

When an unheated tumor is inoculated intraperitoneally first and a tumor which has been heated for thirty minutes inoculated subcutaneously second we find no smaller inhibiting effect of the first intraperitoneal tumor than we would if the first tumor had been inoculated subcutaneously (Table 1). Thus, in the first case the tumor heated for thirty minutes grows in 21 percent, in the second case in 20 percent. Whether the results in these experiments are due to some factors which at present are not evident or whether attributable to coincidence, we cannot state, but even in this case we find the weaker, intraperitoneal tumor at least as active an immunizing agent against a second, subcutaneous tumor as is the much more strongly growing first, subcutaneous tumor.

When five times the usual quantity of tumor material is inoculated intraperitoneally first, a second unheated, subcutaneous tumor is very markedly inhibited, that is, when we compare the effect of the 375 mg. intraperitoneal first with the effect of 75 mg. subcutaneous first. However, the inhibition of the second unheated tumor by 375 mg. of tumor inoculated intraperitoneally is no greater than the effect of the usual 75 mg. inoculated intraperitoneally, even tho the former represents a more actively growing tumor (Table 1).

When the first, intraperitoneal tumor is heated the immunizing effect seems gradually to be lost; the growth of a second tumor heated for twenty-five minutes inoculated subcutaneously following an intraperitoneal tumor which had been heated twenty-five minutes, is better than when an unheated tumor is inoculated first intraperitoneally. When the first, intraperitoneal tumor is heated still longer, viz., thirty minutes, and a tumor heated for thirty minutes is inoculated second subcutaneously, we find that the second tumor has grown better than usual. Therefore, there seems to be a stimulating action produced by the first tumor on the second. On the other hand, the first, intraperitoneal tumor grows in a smaller percentage of the animals than normally, and it seems that the second, subcutaneous tumor has exerted an inhibiting influence on the first, intraperitoneal tumor. We find, therefore, in this case definite evidence of the beneficial action of a tumor of low virulence on a second tumor of low virulence and also an approach toward the mutually exclusive alternating type of growth, inasmuch as the first intraperitoneal tumor grows less than normally. However, as the first tumor grows usually only when the second grows, this alternating type of growth is not fully realized. It is probable that the second, subcutaneous tumor grows more actively under these conditions because the subcutaneous tissue is a better soil for tumor growth than the peritoneum. Furthermore, we must consider that at the time when the second tumor heated for thirty minutes is inoculated subcutaneously the first, intraperitoneal tumor has hardly started to grow, so that the second, subcutaneous tumor has time to begin growth and become established before the full, inhibiting effect of the intraperitoneal tumor develops. When the first tumor is inoculated intraperitoneally and the second tumor subcutaneously, there is no effect of either heated or unheated second tumor on the first tumor, either unheated or heated twenty-five minutes, in a similar manner as a second, subcutaneous tumor has no action on a first, subcutaneous tumor. We have discussed the action of a second tumor on a first tumor heated thirty minutes.

TABLE 2
ANALYSIS OF TAKES

Retrogressions of the Double Inoculations	(1) Tumor Subcutaneous (75 mg., Unheated), (2) Tumor, Subcutaneous			(1) Tumor Subcutaneous (75 mg., Unheated), (2) Tumor, Intraperitoneal		
	(2) 75 mg. Unheated	(2) 75 mg. Heated 25′	(2) 75 mg. Heated 30′	(2) 75 mg. Unheated	(2) 75 mg. Heated 25′	(2) 75 mg. Heated 30′
First and second tumors grew	19	21	6	8	3	8
First tumor grew; second did not grow	..	11	7	22	16	24
First tumor grew; second retrogressed	..	..	6	3	..	..
First tumor retrogressed; second grew	..	..	..	..	..	..
First tumor retrogressed; second did not grow	2	3	..	4	4	1
First tumor retrogressed; second retrogressed	1	16	7	..	1	..
First tumor did not grow; second grew	..	..	..	..	..	..
First tumor did not grow; second did not grow	1	4	4	5	1	5
First tumor did not grow; second retrogressed	..	..	..	1	..	..

INFLUENCE OF A FIRST, SUBCUTANEOUS TUMOR ON A SECOND, INTRAPERITONEAL TUMOR

When an unheated, therefore an actively growing, subcutaneous tumor has been inoculated first, we find that the second, intraperitoneal tumor is inhibited in its growth. The unheated tumors are inhibited as much as the heated tumors (the low percentage of growth in the case of the tumor heated for twenty-five minutes, inoculated intraperitoneally may be due to some accidental condition) ; relatively however, the unheated, intraperitoneal tumors are inhibited more by the first, subcutaneous tumor than are the heated tumors. Thus, while the unheated, intraperitoneal tumor usually grows in 53 percent and the tumors heated for twenty-five and thirty minutes, in respectively 43 and 34 per cent of the animals, when these tumors are inoculated after a first, subcutaneous tumor they grow respectively in 19, 12, and 21 percent of the animals (Table 1). We have reported the same observation in the case of a combination of first, intraperitoneal and second, subcutaneous tumors. The first, subcutaneous tumor inhibits the growth of a second, unheated tumor, or intraperitoneal tumor heated for twenty-five minutes, relatively more than it does the growth of similar, subcutaneous tumors, in accordance with the weaker growth of intraperitoneal tumors generally (Table 1).

TABLE 2
ANALYSIS OF TAKES

(1) Tumor Intraperitoneal (75 mg., Unheated), (2) Tumor, Subcutaneous			(1) Tumor (75 mg., Unheated), Subcutaneous (2) Tumor (375 mg., Unheated), Intraperitoneal	(1) Tumor (375 mg., Unheated), Intraperitoneal (2) Tumor (75 mg., Unheated), Subcutaneous	(1) Tumor Heated 25′; (2) Tumor Heated 25′		(1) Tumor Heated 30′; (2) Tumor Heated 30′	
(2) 75 mg. Unheated	(2) 75 mg. Heated 25′	(2) 75 mg. Heated 30′			(1) Subcutaneous (2) Subcutaneous	(1) Intraperitoneal (2) Subcutaneous	(1) Subcutaneous (2) Subcutaneous	(1) Intraperitoneal (2) Subcutaneous
14	10	8	12	14	17	16	5	15
15	11	6	8	13	1	9	7	4
..	..	1	3	4	1	1	5	..
..	..	..	..	..	..	..	1	4
4	9	4	6	8	4	8	1	2
..	..	..	9	..	1	..	2	5
1	..	..	..	..	..	1	..	26
30	14	22	9	16	3	19	..	18
..	1	..	..	2	1	..	..	..

In all cases, the second, intraperitoneal tumor grows only when the first, subcutaneous tumor grows, and, with the exception of the case about to be discussed, exerts no retarding influence on the first subcutaneous tumor.

Such a retarding influence on the first tumor is noticeable under the following condition: When five pieces (375 mg.) of unheated tumor material are inoculated intraperitoneally, six days after the subcutaneous inoculation of one piece (75 mg.) of unheated tumor material, we find that, even tho the growth of the intraperitoneal tumor is diminished, it exerts an inhibiting action on the growth of the first, subcutaneous piece. On the other hand, the effect of the subcutaneous tumor on the five pieces is probably less marked than on one piece inoculated intraperitoneally.

We must note especially that even in cases in which the second, intraperitoneal tumor did not grow it exerted an inhibiting influence on the first tumor. Furthermore, there must already be present some immunizing effect on the part of the second tumor at a time even before the second shows any growth. It is evident, however, from the larger percentage of growth of the first tumor that the subcutaneous tumor is more resistant to the immunizing than the second, intraperitoneal tumor. The inhibition of growth of the first tumor must react on the second intraperitoneal tumor and further tend to suppress its growth indirectly.

There are three possibilities of interaction of two such tumors: First, the inoculated animal may be to such a degree naturally resistant that the growth of both tumors is suppressed. Second, a slight degree of natural resistance (not sufficient to inhibit an actively growing tumor) may be added to the inhibiting effect exerted by the second tumor on the first, as well as by the first on the second tumor. As a result of these influences either both tumors, or only the second may be prevented from growth. Third, the combined action of a weak, natural resistance and the inhibiting action of both tumors on each other may not be sufficient to prevent the growth of either the first or the second tumor. We find (Table 2) that all three of these eventualities were realized.

When we compare the influence of a first, subcutaneous tumor on a second, intraperitoneal tumor with the influence of a first, intraperitoneal tumor on a second, subcutaneous tumor, we find that relatively (comparing the percentage of growth of each tumor when inoculated second with the normal percentage of growth in case of a single inoculation) a second, intraperitoneal tumor is inhibited to a smaller extent by the first, subcutaneous tumor than a second, subcutaneous tumor by a first, intraperitoneal tumor. It is therefore evident that the inhibiting influence exerted by an intraperitoneal tumor is greater than that of a subcutaneous tumor, and that, even tho a second, intraperitoneal tumor grows in a smaller percentage than a second, subcutaneous tumor, a second, subcutaneous tumor is relatively more inhibited in its growth than a second, intraperitoneal tumor (Table 1).

Krauss, Ranzi, and Ehrlich,[2] who found in their experiments that an intraperitoneal tumor was a more actively growing one than a subcutaneous tumor, came to the conclusion that the intraperitoneal tumor immunized more actively against a second inoculation than did a subcutaneous tumor. Our results agree to some extent with those of these investigators, in as far as they found that intraperitoneal tumors immunized more actively than did subcutaneous tumors. Since they had found in their experiments that the intraperitoneal tumor was the more actively growing, their conclusion was in agreement with the results which had been obtained by Loeb[1] and others to the effect that an actively growing tumor has greater immunizing powers than a less virulent tumor. We have, however, shown in our experiments that even using a tumor which, when inoculated intraperitoneally, grows less actively than when inoculated subcutaneously, we were still able to demonstrate the greater immunizing effect of the weaker, intraperitoneal tumor. Therefore, it appears that the greater immunizing power of the intraperitoneal tumor is due to the location of the tumor and not because it is a more actively growing tumor.

Krauss, Ranzi, and Ehrlich state also that an intraperitoneal tumor caused general immunity, while a subcutaneous tumor caused only local immunity against a subcutaneous tumor but not against an intraperitoneal tumor. We find that both intraperitoneal and subcutaneous tumors exert a general immunity, but that the immunizing effect of the intraperitoneal tumor is stronger. The fact that they used a different tumor from that which we used and that they did not vary the virulence of their tumor may account for the differences in results.

We have also observed the rate of growth as influenced by the double inoculations. Since it is difficult to determine definitely the size of intraperitoneal tumors by palpation and owing to the relatively small number of mice examined in certain cases, we hesitate to draw any definite conclusions from these observations. If, however, in the future opportunity arises to extend these observations we hope to publish these results. We may state that such observations as have been made do not change or affect the conclusions drawn from the observations made on the percentage of takes.

SUMMARY

The number of takes after intraperitoneal inoculation is smaller than after subcutaneous inoculation. After inoculations of unheated (virulent) as well as of heated tumors (less virulent), the number of takes is approximately 40 percent less in the case of intraperitoneal inoculation as compared to the number of growing, subcutaneous tumors treated in the same manner before inoculation.

Notwithstanding the diminished number of takes, the immunizing effect of intraperitoneal tumors is markedly greater than that of subcutaneous tumors, altho the subcutaneous tumors have likewise a decided inhibiting effect on the growth of intraperitoneal tumors.

In a similar manner as the subcutaneous tumor with markedly diminished virulence may exert a favorable influence on another subcutaneous tumor of diminished virulence, a subcutaneous and intraperitoneal tumor may under certain conditions exert a favorable influence on each other as shown by the number of successful inoculations.

The immunizing effect of a first unheated, intraperitoneal tumor on a second, subcutaneous tumor is somewhat greater if the second, subcutaneous tumor had been heated, but even so the inhibiting effect on a more actively growing unheated, second subcutaneous tumor is as evident as on a heated, second tumor. Second, heated, subcutaneous tumors grow almost as well as second, unheated, subcutaneous tumors when in combination with a first intraperitoneal tumor.

If, on the other hand, the first, intraperitoneal tumor is heated it gradually loses its inhibiting power and even a favorable action of the first, heated, intraperitoneal tumor on a second, subcutaneous tumor becomes the more apparent the more the second tumor has been heated.

If the second subcutaneous tumor has been heated only twenty-five minutes the favoring action of a first, intraperitoneal tumor, heated for thirty minutes, is not marked. But when a second, subcutaneous tumor, heated for thirty minutes, follows an intraperitoneal tumor heated for thirty minutes, the second tumor is favored while the first is slightly inhibited. When both tumors are subcutaneous, the first tumor has the advantage of time and thus grows better than normal, while the second is now inhibited. Therefore we observe here similar phenomena as those described in our first communication.[1]

A second, subcutaneous tumor has usually no inhibiting action on any first tumor, whether it is subcutaneous or intraperitoneal, except-

ing possibly the inhibiting action on the part of a second tumor in the case mentioned above, of the two tumors heated for thirty minutes.

A first unheated, subcutaneous tumor inhibits a second, intraperitoneal tumor markedly; it has, however, slightly less inhibiting power on a second tumor after 375 mg. have been inoculated intraperitoneally second than after inoculation of 75 mg.

A first unheated, subcutaneous tumor has a relatively smaller inhibiting effect on a second, heated (twenty-five or thirty minutes), intraperitoneal tumor than on a second, unheated, intraperitoneal tumor. If both tumors were subcutaneous, the inhibiting effect on the second, heated tumor would be greater, while if the second were a unheated subcutaneous tumor the inhibiting effect of a first, unheated subcutaneous tumor would be very slight.

If a large quantity of a second, unheated, intraperitoneal tumor is inoculated (375 mg.) it may even inhibit a first, unheated, subcutaneous tumor. Intraperitoneal inoculation of smaller quantities has no such effect. A first intraperitoneal tumor inhibits a second subcutaneous tumor even in cases in which itself does not grow.

Therefore, we may conclude that both intraperitoneal as well as subcutaneous tumors have a mutual inhibiting action on each other if they are virulent or moderately virulent, but that an intraperitoneal tumor has a relatively stronger inhibiting power than a subcutaneous tumor. The greater inhibiting action of an intraperitoneal tumor on a subcutaneous tumor appears when compared with the action of a first, subcutaneous tumor on a second, subcutaneous tumor, as well as on a second, intraperitoneal tumor. A second intraperitoneal tumor is relatively more strongly inhibited by a first, subcutaneous tumor than is a second, subcutaneous tumor.